# NUTRITIONAL STATUS INFLUENCES BEHAVIOR, PHYSIOLOGY, AND BRAIN GENE EXPRESSION IN PRIMITIVELY EUSOCIAL *POLISTES METRICUS* PAPER WASPS

BY

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THESIS

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**Abstract**

Nutrition is known to influence the division of labor in advanced eusocial insects such as the honey bee, but it is not known whether differences in nutrition can influence the social behavior of primitively eusocial insects such as *Polistes metricus* paper wasp. This is an interesting consideration because if the related behaviors displayed by these two independently evolved social insects are mediated by similar pathways, it may give more insight into the evolution of eusociality. We tested the effect of nutrition on worker division of labor in primitively eusocial insects at three different levels: behavior, physiology, and brain gene expression. In order to understand the effects of nutrition, I manipulated the amount of nutrients received by *Polistes metricus* wasp colonies in the laboratory. The starved group received less prey and sugar during a limited time frame while the fed group received a normal amount of prey and unlimited sugar source. Starvation was found to have large effects on behavior, causing a significantly elevated number of foraging trips per colony after the treatment began. The mean number of foraging trips per day for individual starved wasps was also significantly higher than for fed wasps. Starvation also caused a slightly significant increase in the proportion of foragers in a colony. At the physiological level, individuals from starved colonies had significantly lower abdominal lipid stores than individuals from fed colonies. In addition, there was a significant negative correlation between abdominal lipid and foraging activity for individual wasps in starved, but not fed colonies, showing that individuals with greatly reduced lipid stores foraged at a much higher level. In order to infer the degree of conservation of gene expression influencing worker division of labor, I analyzed brain expression for 24 genes known to be associated with division of labor in honey bees. Some genes showed expression trends similar to that of honey bees while other genes showed different expression trends. The molecular data

provide further support for the idea of a "genetic toolkit" for eusocial behavior because novel regulation of these conserved genes contributes to new forms of related behaviors. The results of this study demonstrate that nutrition has an important role in *Polistes metricus* paper wasps at three different levels: behavior, physiology, and brain gene expression.

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**Table of Contents**

## Chapter 1: Introduction

A defining trait of eusocial insects is a well developed division of labor (Wilson, 1971). Social insects show two forms of division of labor: a division of labor between queens and workers for reproduction and a division of labor among workers for colony tasks (Robinson, 1992). Primitively eusocial insects, such as the *Polistes metricus* paper wasp, differ from highly eusocial species because, first, there are no morphological differences between reproductive and nonreproductive individuals. Secondly, individuals exhibit less defined boundaries between social tasks (Wilson, 1971). For example, *Polistes* workers have retained the ability to mate and reproduce. Understanding whether division of labor in primitively eusocial species is regulated by the same mechanisms as advanced eusocial species could give more insight into the evolution of these societies.

Nutrition is known to influence the worker division of labor in eusocial insect societies but more is known in advanced eusocial societies. An association between nutritional state and worker division of labor has been found in many advanced eusocial species (reviewed in Toth and Robinson, 2005; Toth et al., 2009). Wasps diverged from bees approximately 100-150 million years ago and paper wasps evolved eusociality independently than bees (Danforth et al., 2004). The related social behavior of these insects may be mediated by similar pathways despite their independent origins of eusociality. The idea of a "genetic toolkit" for eusocial behavior has been introduced to explain the conserved set of genes altered throughout the course of evolution to create new forms of behavior (Toth et al., 2007; 2010).

Nutritional effects on worker division of labor in primitively eusocial insects is an interesting consideration because unlike advanced eusocial honey bee workers who have no ability to reproduce, the retention of this ability in primitively eusocial *Polistes* workers creates a

potential conflict: whether to forage for the benefit of the colony or for the benefit of themselves in order to enhance their nutrition for reproduction. This key difference between honey bee and *Polistes metricus* workers gives support to the "genetic toolkit" for eusocial behavior because the same genes are not just being used; they are being used in unique ways. This study focuses on three levels at which nutrition may affect worker division of labor: behavior, physiology, and brain gene expression.

Basic differences in physiology have been found to be associated with worker division of labor in the eusocial honey bee, *Apis mellifera*, by looking at abdominal lipid stores. Honey bee foragers were found to have significantly lower lipid levels than that of nurse bees which do not forage (Toth and Robinson, 2005). Another experiment with honey bees tested for changes in behavior after treatment with TOFA, a fatty acid synthesis inhibitor. Honey bees that had received this treatment were more likely to precociously forage, implying that the depletion of fat stores in a nurse bee causes the transition into a forager (Toth et al., 2005). Food shortage in honey bee colonies has also been reported to cause an acceleration in behavioral development (Schulz et al., 1998). Genome-wide expression patterns in the brain have also been found associated with worker division of labor in honey bees (Whitfield et al., 2003, 2006; Cash et al., 2005).

It is not known whether nutrition can influence the physiology, social behavior, or brain gene expression of worker *Polistes* wasps. Basic differences have been found in abdominal lipid stores in adults suggesting that there is a correlation between nutrition and division of labor in *Polistes metricus* paper wasps. Foraging wasps (foundresses, workers) had lipid stores that were lower than that of nonforaging wasps (queens, gynes) (Toth et al., 2009). Gynes, which typically do not forage, had significantly higher lipid stores than that of workers (Toth et al., 2009).

Molecular mechanisms that influence behavior have begun to be studied in *Polistes metricus*. Genome wide analysis of gene expression in *Polistes metricus* paper wasps and advanced eusocial honey bees, *Apis mellifera*, suggest several common molecular pathways for foraging division of labor (Toth et al., 2007; 2010). These studies, however, do not focus on nutrition or worker division of labor.

To determine whether nutrition has an effect on physiology and social behavior in *Polistes metricus* wasps, I starved colonies to measure abdominal lipid stores and observe the effects on individual behavior. By doing so, I acquired a great deal of information about each individual in the colony, both physiological and behavioral, which could be used to analyze the effect of nutrition on both the colony and individual behavior. In a study similar to the one I conducted, starved honey bee colonies showed a significantly higher proportion of foraging bees than fed colonies (Schulz et al., 1998). I hypothesized that the starvation treatment would cause increased colony and individual foraging activity.

In order to test the effect of nutrition at the molecular level and infer the degree of conservation of gene expression influencing worker division of labor, I performed comparative brain gene expression analysis on 24 genes suggested to influence division of labor in honey bee and paper wasp studies. Because paper wasps and honey bees evolved social behavior independently, I hypothesized that there would be both similarities and differences in expression patterns of the genes controlling worker foraging behavior.

## Chapter 2: Materials and Methods

### *Effect of Nutrition on Foraging Behavior*

I collected nests from several field sites and reared them in an indoor flight cage in order to perform dietary manipulations to determine the effect on behavior. Half of the colonies received a starvation treatment while the other half, the control colonies, received a normal diet. The individual foraging trips were documented during daily observations.

***Nest Collections –*** Forty-two wasp colonies were brought into the lab from two field sites: Forest Glen County Park in Westville, Il (Vermillion County) and Lake of the Woods County Park in Mahomet, Il (Champaign County). The colonies consisted of *Polistes metricus* paper wasps. Nests were naturally occurring on buildings and other human-made structures. I collected only colonies with pupae and at least 3 or more wasps in order to ensure that there would be enough workers. The wasps and nests were both collected between the hours of 5:00am – 8:00am on July 10, 2007 and July 12, 2007 (Fig 2) by quickly scraping the pedicel with a hive tool and catching them into a net. The nest pedicel was glued with hot glue to wooden board and placed over a Plexiglas™ lab rearing box (described below). The wasps were then anesthetized with carbon dioxide, individually paint-marked, and placed in the rearing box with their nest.

***Lab Rearing Conditions -*** I designed a 28cm x 28cm x 28cm (LxWxH) Plexiglas™ lab rearing boxes (Fig 1) in which each colony was kept for the remainder of the experiment. An additional 10cm x 10cm x 10cm (LxWxH) foraging chamber served as the food receptacle as well as a suitable environment for the wasps for foraging.

Each Plexiglas™ box was placed in an indoor flight chamber which simulates a 15 hour summer day with controlled temperature and humidity. The first of four sets of lights turns on at 5:30 am with the rest following in 15 minute successions after. The first set of lights then shut

off starting at 7:30 pm and following in 15 minute successions. The temperature during the day remained ranged from 31-35°C with a humidity of approximately 50-70%. During the night, the temperature cooled to 20-25°C with a humidity of around 30-33%.

Each nest was inspected every other day to check for newly emerged wasps, eggs, and number of pupae and larvae. When new wasps emerged, the entire colony was anesthetized with carbon dioxide gas to allow for paint marking. All colonies were treated this way so that each colony and its treatment pair received equal carbon dioxide. Wasps were individually marked with different colored paint on their thorax in order to note their date of emergence (+/- 1 day) as well as to facilitate individual identification during behavioral observations.

***Feeding Regimen and Starvation Treatment*** - In order to control for variation in colony size (which ranged from 4 to 28 individuals), each colony was assigned a certain amount of food based on size of colony. Each worker was assigned 0.125g prey/day (approximately ½ a caterpillar) and each larva in the nest 0.010g prey/day (approximately $1/12^{th}$ a caterpillar). Prey, which consisted of either cabbage loopers (*Trichoplusia ni*) or wax worms (*Galleria mellonella*), were cut into the appropriate mass for each individual colony. All colonies on a given day were fed the same prey species. In addition, each colony, regardless of size, was given water and rock candy crystals for a source of sugar. This system was designed in order to provide a normal amount of food per day and was determined from previous dietary observations (A. Toth, personal communication). The diet per colony was recalculated weekly due to new larvae emergence and caterpillar mass was precise to the first two decimal places. If a new adult wasp emerged, additional food was provided mid week accordingly. Every colony received this treatment from the collection date (July 10 or 12, 2007) to July 23, 2007 when starvation treatment began for certain colonies (day 7 of the experiment; Fig 2).

After every nest had a minimum of 3 workers, colonies were paired by similar number of wasps and pupae. One of the colonies in the pair was assigned to receive the starved treatment while the other continued receiving the same normal/control, or fed treatment. This was done in order to ensure that both experimental treatment groups, starved and fed, had roughly the same sample size and the same distribution of colony sizes.

The colonies assigned to the starved group received $1/5^{th}$ the control amount of food for their individual colony while the colonies of the fed group continued with the control prey amount. In addition, to ensure the starved group was starved, they were only allowed to access the prey for 3 hours a day during behavioral observations (as described below). The fed colonies received the normal/control amount of prey along with unlimited rock candy crystals and water. Also, the fed colonies were able to retrieve the food at any point in the 24 hour day. The starvation procedure was performed for 8 days.

***Behavioral Observations*** **-** On days 1-6 (July 17 – July 22) of the experiment, all colonies were given the same normal diet, explained above, so that normal foraging behavior could be observed (Fig 2). Blind observations were done so that accurate records were made of which wasps were foraging, what for (rock candy, prey, or water), and how often. Observation scans were performed in 15 minute intervals during the times of 10:00-11:30am (6 scans) and 1:30-3:00pm (6 scans) by two observers (Amy Toth, T.H.F.D.) On days 7-16 of the experiment, the same behavioral observation protocol was performed, but the rock candy and prey for the starved colonies were removed after the observation periods (Fig 2).

On day 17 (August 2), all colonies were anesthetized with carbon dioxide and collected onto dry ice between 9:30 am – 2:00 pm (Fig 2). Each wasp was put in its own tube and was stored in a -80°C freezer.

***Statistics*** **-** All data were analyzed using the R program. The "Effect of starvation on colony foraging behavior" (Fig 3A) was first tested by looking at the mean number of foraging trips made by all wasps in a colony per day for rock candy only. The data were analyzed using a repeated measures ANOVA, which accounted for colony as a random factor, using the lme function.

The "Effect of the starvation on individual foraging behavior" (Fig 3B) was then tested by looking at the mean number of foraging trips over days 8-16 per individual per day for both groups. I tested for differences in foraging activity between starved and fed individuals (including only individuals who had been seen foraging at least once on rock candy, prey, or water) by performing a two-sample t-Test using a linear model which accounted for colony as a random factor, also using the lme function.

The "Effect of starvation on the proportion of foragers per colony" (Fig 3C) was tested using a two-sample t-Test assuming unequal variances again using a linear model to account for colony as a random factor with the lme function.

***Effect of Nutrition on Physiology***

Lipid analyses were performed on the individuals from the starvation experiment to determine differences in abdominal lipid stores. Because these individuals were from the starvation experiment, nest collections, lab rearing conditions, feeding regimen, and starvation treatment were as described above.

***Selection of Starvation Wasps for Physiological Measurements*** **-** Wasps were chosen for analysis based on emergence date and foraging activity. All analyzed wasps had to have emerged in the field prior to collection (PC = pre-collection emergence). This was done in order

to avoid effects of lab rearing and to prevent gynes, which emerge later in the season, from being analyzed. All PC wasps from all colonies that had been seen foraging at some point during the experiment were chosen for physiological measurement. I wanted to focus on wasps that were competent to forage, so wasps never seen foraging were not included. If a given colony only had one PC wasp seen foraging, however, this wasp was not used for measurement because there was no other colony member to compare it with.

***Abdomen Dissections*** **-** The abdomen of each PC wasp was dissected under a microscope to remove all contents other than fatty tissue. The abdomen was removed from the -80°C freezer and secured in a dissection plate with a pin. Two longitudinal cuts were made into the ventral side of the cuticle of the abdomen and the inside was sprayed with a 100% ethanol solution. Then, the internal organs were gently lifted out with forceps in order to remove everything except for the fatty tissue lining the cuticle. During the dissection, the ovaries were removed and their development was scored using a method I developed similar to one used in honey bees (1=completely developed eggs, 2=slightly developed with <2 completely developed eggs, 3=completely undeveloped, string like) (Velthuis, 1970). Ovaries were measured and documented in order to insure only workers, with ovaries of size 2-3, were used for analysis.

***Lipid Quantification*** **-** Each sample was homogenized in a glass tissue grinder in 2:1 chloroform:methanol solution and extracted overnight in 5 ml of the solution. 12 or more hours later, the sample was run through glass wool, evaporated down using a speed vac, and adjusted to a constant volume of 2 ml (Toth et al., 2009).

Lipid was quantified for each sample by taking 30 μl of the sample and drying it down completely using a Speed Vac, then 0.2 ml concentrated sulfuric acid was added and tubes were placed in a boiling water bath for 10 minutes. After this, 2.0 ml of vanillin reagent (0.6%

vanillin in 85% phosphoric acid) was added to each sample, vortexed, and stored in darkness for 15 minutes to allow the colorimetric reaction to occur. Absorbance of each sample was then measured at 525 nm using a BioTek Synergy HT microplate reader. A standard curve, which was used to calculate the sample lipid amounts with the BioTek Gen5 data analysis software, was set up using measured amounts of cholesterol.

***Statistics*** **-** For the statistical analyses on lipid stores, I removed individuals with an ovary score of 1, which were suspected to be queens. The "Effect of starvation on individual abdominal lipid stores" (Fig 4) was tested using a mixed model ANOVA to determine significance while accounting for colony as a random factor, using the lme function.

I tested for correlation between lipid and foraging activity (Fig 5) using the cor.test using the Pearson correlation option. I determined whether the slopes of the graph were different with an ANOVA.

### *Effect of Nutrition on Brain Gene Expression*

Real time qPCR was done for 24 genes (Table 1) in order to study the molecular mechanisms which respond to this change in nutrition. Because differential gene expression could be caused by nutrition or behavior, two new experimental groups were introduced: field-reared foraging and non-foraging workers. The wasps from the starvation experiment were used to represent gene expression differences due to nutrition because they had received dietary manipulations. The foragers and non-foragers were used to represent differences due to foraging because there were no foraging limitations as there were in the lab. Foragers and non-foragers have been found to be two distinct groups based on significantly different levels of abdominal lipid (Toth, unpublished). mRNA levels in the starved and fed individuals from the starvation

experiment were also analyzed using qRT-PCR. Differential brain gene expression between field-reared foragers and non-foragers offered comparison to the differences in gene expression of the starved and fed wasps.

***Collection of Field-reared Workers -*** Foraging and non-foraging workers were collected from the Vermillion River Observatory in Vermillion County Illinois between the hours of 5am-7am on July 20$^{th}$ 2009. Workers were only collected from colonies with 8 or more total wasps. The wasps were immediately placed onto dry ice following anaesthetization with $CO_2$ and placed in individual tubes for storage in a -80℃ freezer. A total of 36 wasps were collected from 10 different nests: 2 foraging and 2 nonforaging workers were taken from each nest, when possible. Foragers were collected on the basis of having noticeable wing wear while non-foragers were collected without wing wear. All wasps were collected prior to emergence of males also increasing our chances of collecting worker females, rather than gynes, which tend to emerge around the same time as males.

In early June, prior to the emergence of workers, the foundress of each nest was anaesthetized with $CO_2$ for less than a minute and paint-marked on the thorax in order to ensure that only workers were collected on the collection date.

***Selection of Starvation Wasps for Brain Gene Analysis -*** Individuals were chosen for analysis of brain gene expression based on the mean number foraging trips per day of the individual over days 8-16 of the experiment. This was calculated by taking the total number of foraging trips made by that individual over days 8-16 and dividing by 9. In order to be included in this analysis, the individual must have been seen foraging at least once during the last 9 days.

If there were more than two individuals in a given colony that foraged and had also emerged pre-collection (PC), we chose two wasps representing the most and least active foragers

from each colony. Wasps were chosen in this fashion in order to best capture expression differences related to the starvation treatment, independent of differences in foraging activity.

***Genes for Analysis*** **-** Twenty-four genes were selected for analysis of brain gene expression (Table 1). Twenty-one of these genes were selected based on prior expression studies with *Polistes metricus* and were found correlated with provisioning, reproduction, or an interaction between the two (Toth et al. 2007, Toth et al. 2010). Two other peptide encoding genes (*Pmtachykinin* and *PmsNPFR*) were chosen because they are known to be involved in regulating food intake in solitary insects and are associated with nectar and pollen foraging in honey bees (Brockmann et al. 2008). Lastly the gene *PmTOR* was chosen because the TOR nutrient-sensing pathway has been shown to affect the maturational onset of foraging behavior in honey bees (Ament et al. 2008).

***Analysis of Brain Gene Expression*** **-** The heads of the individual *Polistes metricus* workers were freeze dried at -80˚C for 58 minutes and the brains were dissected on dry ice. All surrounding muscle, gland, and fat tissues were carefully removed in order to attempt to isolate only brain tissue. Brain gene expression was measured by analyzing abundance of mRNA per individual using an established protocol (Toth et al. 2007). Outliers with mRNA values greater than 2 standard deviations from the group average were removed prior to statistical tests.

***Statistics*** **-** All statistical analyses were performed with R statistical software. The brain gene expression data were normalized to rcp for all statistical procedures.

The analysis of brain gene expression for field-reared workers was performed using a two-sample unpaired t-test (assuming unequal variance with a linear model to account for colony as a random factor) to compare the relative abundance of mRNA for individual genes in foraging and nonforaging workers.

The analysis of brain gene expression for lab-reared fed and starved workers was performed using a two-sample unpaired t-test (assuming unequal variance with a linear model to account for colony as a random factor) to compare the relative abundance of mRNA.

## Chapter 3: Results

### *Effect of Nutrition on Foraging Behavior*

Nutrition had an effect on foraging behavior. Starvation caused a significantly elevated number of foraging trips per colony over the last 9 days versus the first 6 days of the experiment (repeated measures ANOVA: day*treatment $F_{1,628}$=97.60404, $P$<0.0001; Fig 3A). This significant difference was driven by the starvation treatment during days 8-16 as seen in comparison to the baseline treatment during days 1-6.

Individual mean foraging trips per day for rock candy, prey, or water (for wasps who had been seen foraging at least once throughout days 1-16) were significantly higher for starved wasps (t-Test: Two-Sample Assuming Unequal Variances: $P$<0.0001, t=4.05; Fig 3B).

In addition to effects on foraging rate, there was also an effect of starvation on number of foragers per colony. The proportion of foraging workers was slightly higher in starved versus fed colonies (t-Test: Two-Sample Assuming Equal Variances: $P$<0.05, t=-2.0838; Fig 3C).

### *Effect of Nutrition on Physiology*

There was no significant difference in abdominal lipid stores between starved and fed wasps for all pre-collection-emergence (PC) individuals (mixed model ANOVA: $F_{1,27}$=0.59, $P$>0.05). However, I noticed that 16 of the wasps had very high lipid stores, which are characteristic of gynes rather than workers. Gynes typically have lipid stores > 4 mg (Toth, et al., 2009), and these 16 individuals all have lipids > 4 mg. Since I was interested in workers, I performed another analysis with individuals with lipid > 4 mg removed and found that the lipid stores of starved wasps were significantly lower than fed wasps (mixed model ANOVA: $F_{1,27}$=8.97, $P$<0.01; Fig 4).

There was a significant negative relationship between abdominal lipid stores and foraging trips per day for individual wasps in starved colonies (Pearson correlation: R= -0.51, $t_{45}$=-4.02, *P*<0.001; Fig 5). For fed colonies, there was no relationship between abdominal lipid stores and foraging trips per day (Pearson correlation: R= 0.002, $t_{41}$=0.013, *P*>0.05; Fig 5). In addition, the slopes for starved and fed wasps were slightly different (ANOVA: $F_1$=3.28, 0.05<*P*<0.01; Fig 5) suggesting that the relationship is only detectable in starved colonies because there are many wasps with very low lipid stores.

The foraging activity results reported above were calculated after removing the individuals with high lipid amount (lipid>4mg). The mean foraging trips per day for the individuals with high lipid amount was 0.58 ± 0.19 trips/day, whereas those with lower lipid amount made 3.31 ± 0.29 trips/day. The foraging rate for high-lipid individuals was significantly lower than that for lower-lipid individuals (t-test: *P*<0.0001).

***Effect of Nutrition on Brain Gene Expression***

Twenty-four genes were analyzed for differential expression between the two experiments (Fig 7). Of these, 6 genes were differentially expressed between both the starved and fed groups as well as the forager and non-forager groups. These genes were *PmSPARC, Pmtun, PmKul, Pmusp, PmILP2,* and *PmCG11971*. Of these genes, *PmSPARC, Pmtun, Pmusp, and PmILP2* were all upregulated in fed wasps and non-foragers. *PmCG11971-like* was upregulated in starved wasps and foragers. *PmKul* was upregulated in fed wasps and foragers.

An additional 4 genes were found significantly different between the starved and fed groups but not between foraging and non-foraging workers. These genes were *PmsNPFR,*

*PmTachykinin*, *PmInR2,* and *PmVg1*. Three of these genes, *PmsNPFR, PmTachykinin, and PmInR2,* were upregulated in starved wasps while *PmVg1* was upregulated in fed.

Finally, another 4 were significantly different between foraging and non-foraging workers but showed no significant difference in expression between the starved and fed groups: *PmRfaBp, PmSh3Beta, PmTOR*, and *Pmoxidoreductase*. All of these genes were upregulated in foragers.

## Chapter 4: Discussion

The results from this study show that nutrition is an important factor in *Polistes metricus* worker division of labor at three different levels: behavioral, physiological, and molecular. The mechanisms influencing foraging behavior in primitively eusocial insects at the behavioral and physiological levels are similar to the mechanisms in advanced eusocial insects. In *Polistes metricus*, colonies responded to starvation treatment by a large increase in foraging behavior. The data from my experiment allowed me to distinguish between two potential causes for this increase: the starved colonies could be producing more foragers, or the individuals within the colonies that were already foraging could forage more frequently. I found that individual starved wasps foraged significantly more per day than fed wasps, whereas the number of foragers from starved colonies was only slightly more significant than fed colonies. This indicates that wasps that were already foraging prior to the start of the treatment began foraging more frequently after the starvation treatment began and the treatment did not cause many new wasps to begin foraging.

In addition, the results on individual behavior and physiology suggest that extremely low abdominal lipid stores are associated with elevated foraging. I found a negative relationship between the individual amounts of abdominal lipid stores and foraging activity that I expected to see in the starved colonies, however, there was no relationship for fed colonies. The dramatic relationship seen in the starved wasps is strongly affected by the fact that many wasps from the starved colonies had abdominal lipid stores below 1 mg. The relationship for fed colonies may not have been captured for two possible reasons: non-linear relationship between lipid and foraging and the effects of lab rearing conditions.

It is unlikely that the lack of relationship between the amount of abdominal lipid and individual foraging activity in fed wasps is due to lab rearing effects. It has been shown in other species that in nature, workers expend a great amount of energy in order to fly from the nest on a foraging trip, reducing their abdominal lipid stores (Markiewicz and O'Donnell, 2001; Toth and Robinson, 2005). In the lab, however, one might suspect that the foraging process may be less energy expensive because the food is stored in the colony's rearing chamber; because the fed wasps may not have been burning as much energy during foraging trips, this could have caused their abdominal lipids to remain relatively high. However, because the fed lab-reared wasps ($1.53 \pm 0.12$ mg) had similar abdominal lipid levels to that of the typical field-reared worker (between 1.5-2.0 mg; reviewed in Toth et al., 2009) the lack of relationship seen in fed colonies was most likely not caused by rearing condition.

Because a correlation was found in starved but not fed wasps, it is more likely that the relationship between abdominal lipid stores and foraging behavior is not linear; there were more wasps with depleted abdominal lipid stores (< 1 mg) in starved colonies, and these wasps foraged much more than fed wasps. This suggests there may be a threshold lipid level which causes the individual to forage at a very high rate.

It is clear that foraging behavior is intimately related to dominance in the eusocial *Polistes* wasps (O'Donnell, 1998) and this has also been found in other species. The effect of nutrition on dominance has been studied in the eusocial wasp *Mischocyttarus mastigophorus*, leading to the development of a model called the "dominance-nutrition hypothesis." This model proposes a cycle that shows that the nutritional costs and benefits of certain social tasks in primitively eusocial species determine ovary development, which then determines dominance behavior (Markiewicz & O'Donnell, 2001; reproduced in Fig 6). The results from this starvation

study do not disprove this theory, but rather suggest the cycle is incomplete because task performance can also be influenced by nutritional state (Fig 6). The rearing conditions of this study further support this new element to the dominance-nutrition model. Because the workers in the lab do not likely burn as much energy in a foraging trip, the change in abdominal lipid for starved individuals is most likely due to the nutritional treatment and not task performance.

Sixteen of the individuals I analyzed had extremely high amounts abdominal lipid stores, very low levels of foraging activity, and completely undeveloped ovaries. These physiological characteristics are typical to gynes. Gynes, which in temperate *Polistes* species emerge later in the season, are the individuals that overwinter to become the foundresses the following spring and they typically refrain from energy expensive tasks such as foraging. In addition, gynes have abdominal lipid stores much higher than those of any other group (around 4 mg) as well as the smallest ovaries (Toth et al., 2009). Because I intended to study workers, this was an interesting finding because lipid quantification was done on all wasps that had emerged before collection on July 10, 2007 and July 12, 2007. This suggests that there are gyne-like females on the nest during the worker phase of the colony, which could mean the worker and reproductive phases of the colony are more similar to a continuum than two distinct phases.

In addition, because these gyne-like females were found to have extremely low levels of foraging, the data provide additional support for my hypothesis that nutritional status affects foraging behavior. Although the primary focus of the study was on worker behavior, these data reinforce the negative relationship between nutrition and foraging behavior: the higher abdominal lipid stores, the less the wasp forages.

Nutrition also had an effect at the molecular level. Several genes associated with behavior in the honey bee showed similar expression trends in *Polistes metricus* paper wasps

while some showed different trends. Of the 24 genes examined, 6 were significantly different between starved and fed and also field-reared foragers and non-foragers. In 5 out of these 6 genes, expression was in the predicted direction: Starved wasps were more similar to field-reared foragers based on relative abundance of mRNA. This suggests that regulation of these genes is related to both foraging and nutrition. These genes may link foraging with nutrition by responding to the change in nutrition and causing a change in behavior. In honey bees, the transition from a non-foraging nurse to a forager has been shown to be associated with a decrease in abdominal lipid stores and an alteration in brain expression of nutrition related genes. *SPARC* is upregulated in these non-foraging nurses suggesting that this gene, involved in cell adhesion, may cause changes in brain structure which assist in the transition to foraging behavior (Whitfield et al., 2003). *SPARC* is also upregulated in both non-foraging and well nourished *Polistes* workers, suggesting that *SPARC* may also link nutritional state to foraging in these primitively eusocial wasps.

The insulin signaling pathway has been implicated as a regulator of both reproductive and worker division of labor in honey bees (Ament et al., 2008). The insulin like peptide encoding gene, *ILP2*, is upregulated in inactive honey bee foragers compared to bees collected while foraging (Naeger & Robinson, unpublished) which means this gene may be mediated by short term foraging activity. *PmILP2* may also link nutritional physiology and foraging behavior in *Polistes metricus* workers. *ILP2* is negatively correlated with ovary development (Toth et al., 2009) which suggests there could potentially be a relationship between *PmILP2* and nutrient allocation. In this study we found elevated levels of *ILP2* in non-foragers and fed individuals. Therefore we hypothesize that in worker *Polistes metricus*, *ILP2* may be upregulated in response to increased nutritional state and subsequently repress a foraging response. The insulin receptor,

*PmInR2*, was significantly upregulated in the brains of starved wasps but there were no significant differences between foragers and non-foragers suggesting the receptor is associated with nutrition. Similar to honey bee studies (Ament et al., 2008), the results from this study also suggest that insulin signaling is also involved in regulation of worker division of labor in *Polistes metricus*.

Many studies of division of labor in insects focus on the role of juvenile hormone (JH) as a regulator of developmental and physiological processes. Treatment of honey bee workers with JH caused foraging to begin at a younger age, thus implicating JH as a mediator of temporal polyethism in advanced eusocial insects (Robinson, 1987). The role of JH seems to vary in advanced and primitively eusocial insects. It has been proposed that throughout social evolution the role of JH has changed in advanced eusocial insects to regulate worker division of labor after worker reproductive function was lost (Robinson et al., 1992). In primitively eusocial insects, however, it was previously thought that JH influences only aggression and dominance. More recently, treatment of *Polistes dominulus* workers with JH has also shown to accelerate the onset of foraging behavior therefore suggesting that JH may also mediate temporal polyethism in some primitively eusocial insects as well (Shorter & Tibbetts, 2008). Ultraspiracle protein (USP) as well as other nuclear receptors and cofactors are known to help mediate transcriptional regulation of JH associated genes in *Apis mellifera* (Li et al., 2007). In this study, *Pmusp* was upregulated in non-foragers and fed wasps. Differential expression of the JH associated USP between these two experimental groups in this study also suggests that JH may have a role in linking foraging with nutrition by mediating the behavioral transition to foraging in primitively eusocial *Polistes metricus* wasps.

Four other genes (*PmsNPFR, PmTachykinin, PmInR2*, and *PmVg1*) were differentially expressed between starved and fed workers but not between field-reared foragers and non-foragers. It is likely that these genes are nutritionally related in *Polistes metricus* because the nutritional manipulation induced lower lipid stores in starved wasps which also show differential gene expression. Tachykinin and sNPFR (the receptor for sNPF) are both involved in neuropeptide signaling pathways which are known to regulate feeding behavior in solitary insects (Melcher et al., 2006; Melcher & Pankratz, 2005). In honey bees, no significant difference in expression of these genes has been found between nurses and foragers, however, there is evidence that these peptides are involved in a short term predisposition of foraging for nectar and pollen (Brockmann et al., 2008). Because these genes appear to be associated with nutrition in primitively eusocial *Polistes metricus* wasps, this result provides further support for the idea that social evolution may have modified their function to be involved in one or more aspects of honey bee social foraging (Brockmann et al., 2008).

Vitellogenin (VG) is a yolk protein known associated with reproductive physiology in honeybees. Honey bee queens express higher levels of VG than workers in the abdomen and fat body cells of the head and thorax (Corona et al., 2007). Differential expression has also been noted in workers with nurse bees showing elevated expression of abdominal VG relative to that of foragers (Amdam et al., 2003). VG has also been shown to help regulate foraging behavior in honey bee workers (Amdam et al., 2006). In *Polistes metricus*, it is important to note that VG has shown evidence of expression in non-brain tissue in the head (Toth et al., 2007). Despite this fact the results from this study show an interesting connection between nutrition and Vitellogenin. The gene *PmVg1* has been found positively correlated with ovary development and closely reflects reproductive behavior but does not seem to be involved in foraging behavior

in *Polistes metricus* (Toth et al., 2007). This study provides further evidence that vitellogenin may retain its ancestral reproductive role because it does not appear to be involved in foraging behavior (Toth et al., 2007). Because it was found associated with nutrition in this study and a positive correlation has previously been found with ovary development it is possible that vitellogenin may be linked with reproductive capacity in this species.

Expression levels for 4 other genes (*PmRfaBp, PmSh3Beta, PmTOR, Pmoxidoreductase*) were significantly different between the field-reared foragers and non-foragers but not between starved and fed groups. It is likely that expression of these genes is not related to nutrition because significant differences were not found in starved and fed groups. Therefore, it is more likely that these genes are associated with foraging.

Oxidoreductase activity is significantly upregulated in foraging honey bee workers as compared with non-foraging nurses (Whitfield et al., 2006). In prior gene expression studies with *P. metricus* oxidoreductase has been associated with provisioning tasks (Toth et al., 2007). The results from this study lend support to previous findings by showing upregulation of oxidoreductase in foraging workers suggesting that this gene may play a role in mediating foraging behavior in *P. metricus* worker division of labor.

*PmSh3Beta* and *PmRfaBp* are two other metabolic genes that also seem to be associated with foraging rather than nutrition. Both of these genes are differentially expressed in worker division of labor in the advanced eusocial honey bee (Whitfield et al., 2006) and have been found associated with provisioning in *P. metricus* (Toth et al., 2007). Brain gene expression of *PmSh3Beta* has also been found negatively correlated with abdominal lipid (Toth et al., 2009). Results from this experiment show that foragers have higher expression of this gene than do non-foragers. Changes in foraging behavior may lead to changes in brain *Sh3Beta* expression which

subsequently leads to a physiological change. The retinoid- and fatty acid-binding protein, which plays a role in lipid transport and metabolism, is also upregulated in field reared foragers and seems to play a similar role in response to foraging behavior.

The TOR nutrient-sensing pathway, a well studied pathway in honey bees, is upregulated in response to the insulin/insulin-like growth factor signaling pathway and known to influence behavioral maturation. Treatment of honey bees with rapamycin (a TOR inhibitor) causes a delay in the onset of foraging (Ament et al., 2008). *P. metricus* foraging workers showed upregulation of *PmTOR* which indicates the TOR pathway may also be involved in the behavioral maturation of primitively eusocial insect workers.

The results from this study suggest that nutrition has an effect on worker division of labor in primitively eusocial *Polistes metricus* paper wasps at three different levels: behavioral, physiological, and molecular. The effect of nutrition at the behavioral and physiological levels is similar to that of honey bees; starvation resulted in increased foraging behavior in both honey bees and *Polistes metricus* as well as decreased abdominal lipid levels. There are several similarities and differences in how nutrition influences worker division of labor at the molecular level in *Polistes metricus* versus the honey bee. The similar mechanisms highlighted in this study provide further support for the idea that common molecular pathways regulate complex social behavior across social evolution. Mechanisms that differ between the honey bees and paper wasps suggest that the differences between primitive and advanced social behavior could be the result of the novel regulation of these conserved genes.

**Chapter 5: Tables**

| *Polistes metricus* Gene Name | Honey Bee Ortholog |
|---|---|
| *PmSPARC* | GB11432 |
| *Pmtun* | GB12019 |
| *PmKul* | GB19179 |
| *Pmusp* | *Amusp* |
| *PmILP2* | GB10174 |
| *PmCG11971-like* | GB26541 |
| *PmsNPFR* | GB30377 |
| *PmTachykinin* | GB12290 |
| *PmInR2* | *AmInR2* |
| *PmVg1* | *AmVg1* |
| *PmRfaBp* | GB11059 |
| *PmSh3Beta* | GB19996 |
| *PmTOR* | GB11213 |
| *Pmoxidoreductase* | GB14956 |
| *PmInR1* | *AmInR1* |
| *Pmg5sd* | GB15049 |
| *PmGB10722-like* | GB10722 |
| *PmInos* | GB14423 |
| *Pmmcp* | GB18699 |
| *Pmtctp* | GB16412 |
| *PmERK7* | GB11031 |
| *PmCG9005-like* | GB19397 |
| *Pmfor* | *Amfor* |
| *Pmtif2B* | GB12470 |

***Table 1*** The 24 genes analyzed in this study. Honey bee ortholog refers to the best match in the honey bee genome.

**Chapter 6: Figures**

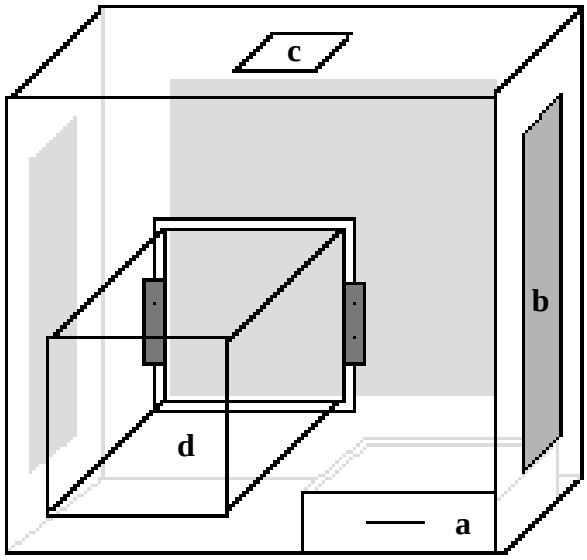


***Figure 1*** Wasp nest container. Each container was equipped with a Plexiglas™ drawer (a) which could be easily opened to provide wasps with rock candy, water, and prey. In addition, the container contained 3 mesh sides (b) for ventilation. The hole on top of the box (c) accommodated the nests which were glued to a wooden board using a hot glue gun and placed over the hole. When foraging observations began, an additional chamber (d) was attached to the front of the box.

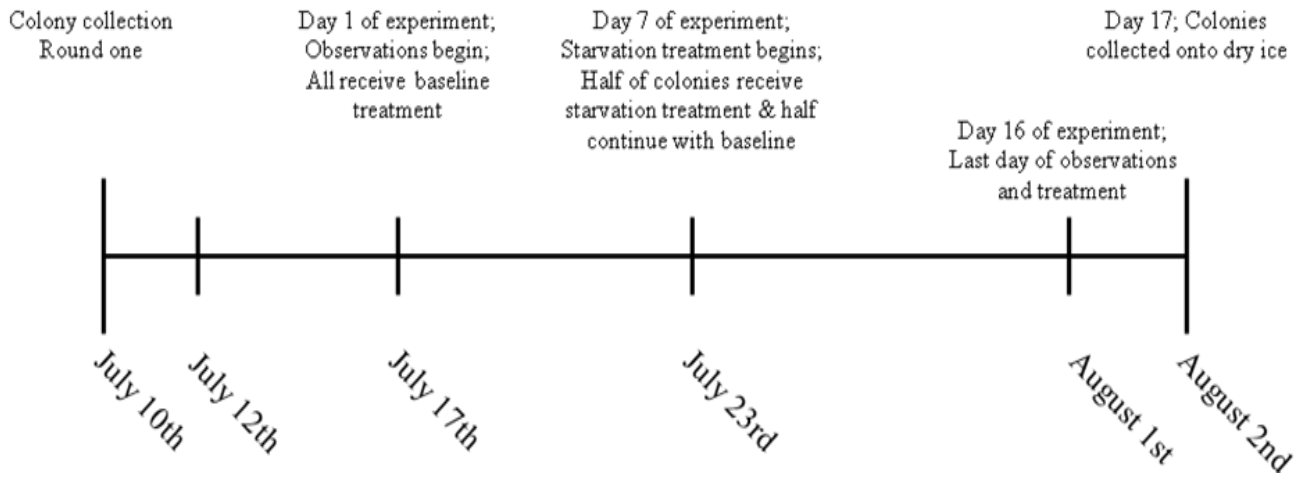


***Figure 2*** Timeline showing the dates and descriptions of daily procedures during the entirety of the experiment.

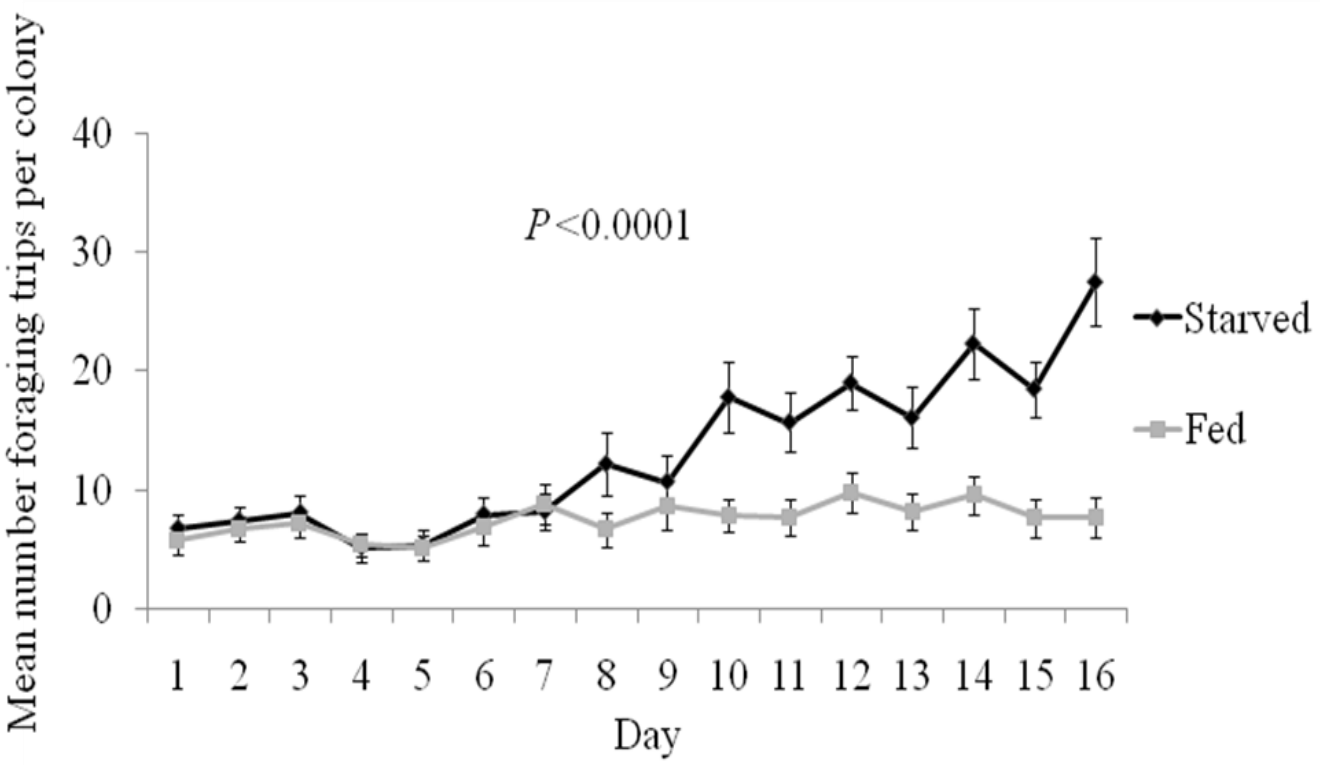


***Figure 3A*** Effect of starvation on colony foraging behavior. The total foraging trips for the individuals in all 42 colonies per day were added and grouped by treatment in order to show the effect of nutrition on foraging behavior. The activity of the starved colonies rose sharply after day 7 when the starvation treatment began. Starved colonies had significantly more foraging trips per colony over the last 9 days versus the first 6 days of the experiment (repeated measures ANOVA: day*treatment $F_{1,628}$=97.60404, $P$<0.0001).

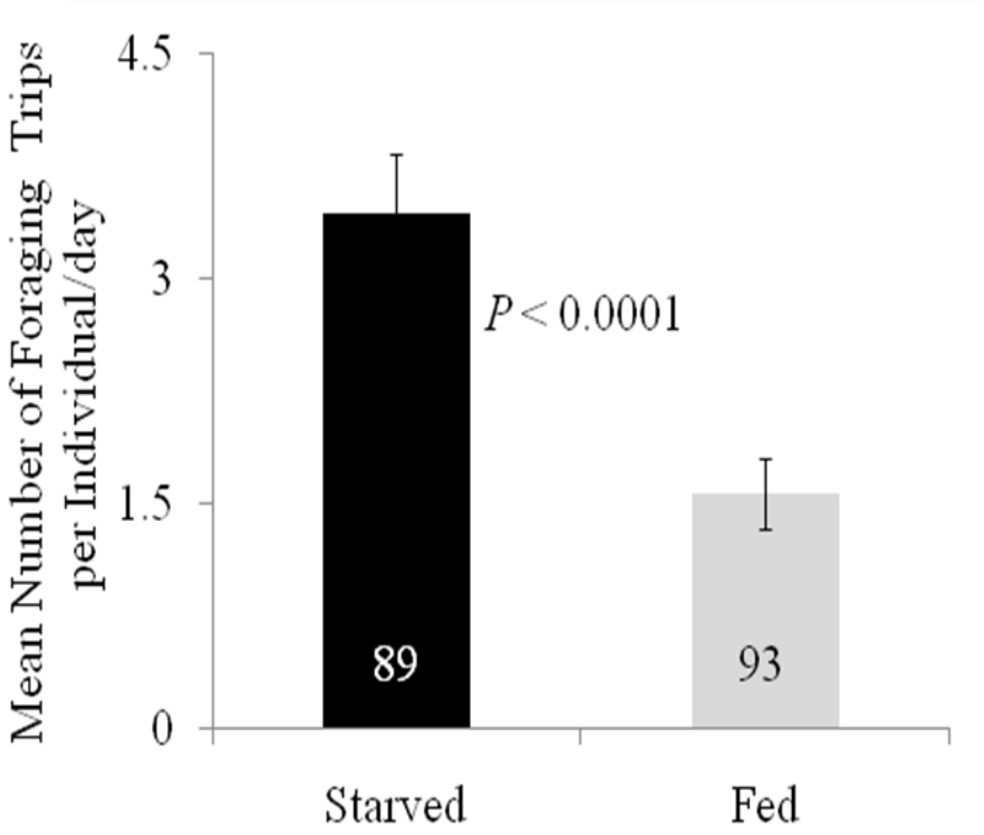


***Figure 3B*** Effect of starvation on individual foraging behavior. Wasps in starved colonies foraged more (t-Test: Two-Sample Assuming Unequal Variances: $P<0.0001$, t=4.05). The total foraging trips per individual over days 8-16 were added and divided by 9, the total number of days after the treatment began. The numbers inside the bars represent the number of wasps observed foraging at least once throughout days 8-16.

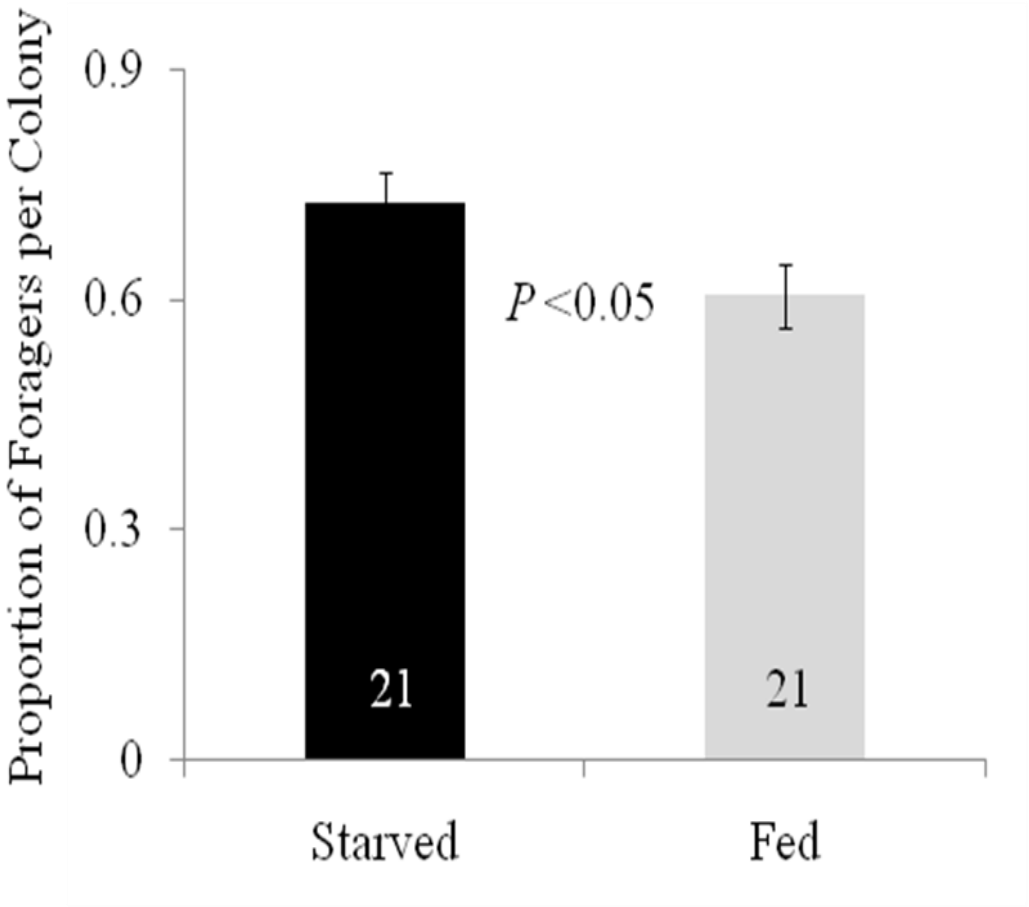


***Figure 3C*** Effect of starvation on the proportion of foragers per colony. Starved colonies had more foragers (t-Test: Two-Sample Assuming Equal Variances: $P<0.05$, t=-2.0838). The number of foraging individuals in a colony was standardized by the total number of wasps on the colony. The numbers inside the bars represent the number of colonies which received each treatment.

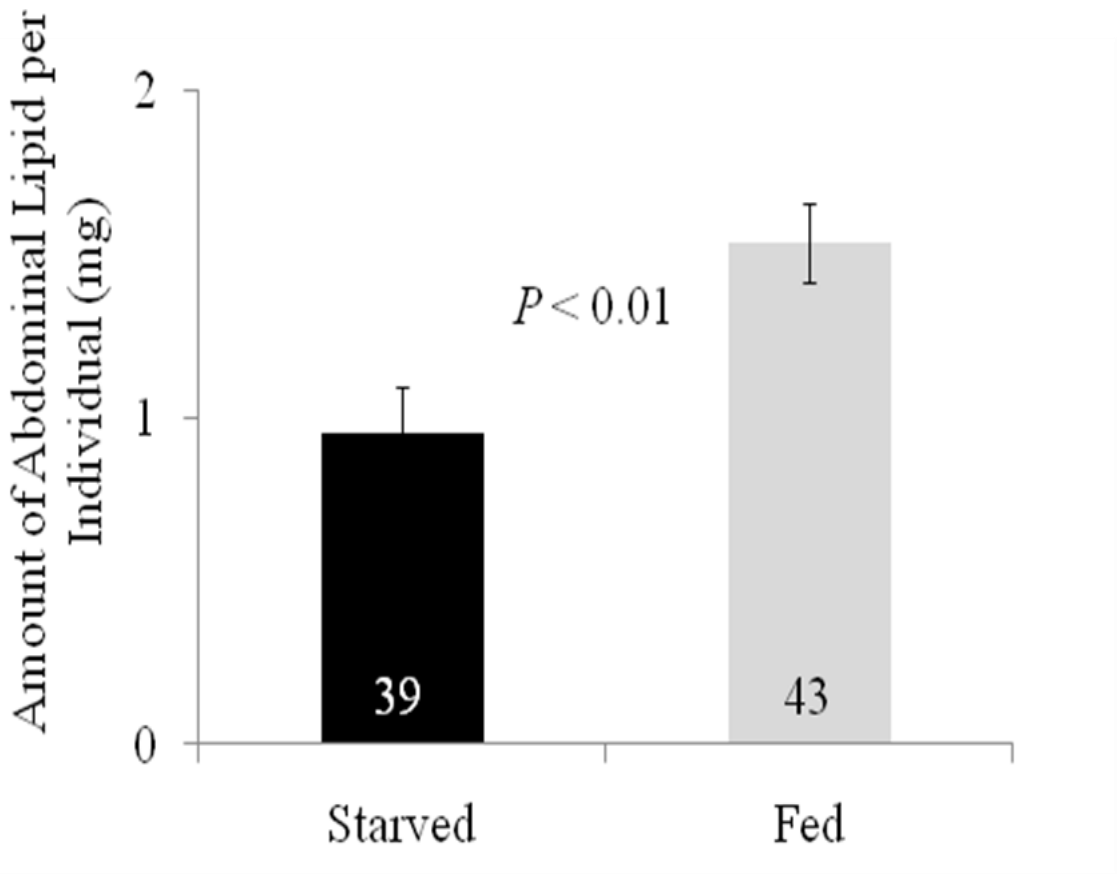


***Figure 4*** Effect of starvation on individual abdominal lipid stores. The mean abdominal lipid amount for all foraging individuals with pre-collection emergence (PC) was lower in starved wasps as compared to fed wasps (mixed model ANOVA: $F_{1,27}$=8.97, $P$<0.01). This shows that the nutritional manipulation was effective and that the increase in foraging found in starved colonies was most likely a result of lower abdominal lipids. The numbers inside the bars represent the number of individuals whose abdominal lipids were quantified. This excludes individuals with large ovaries (typical of queens) and high abdominal lipid amount (typical of gynes).

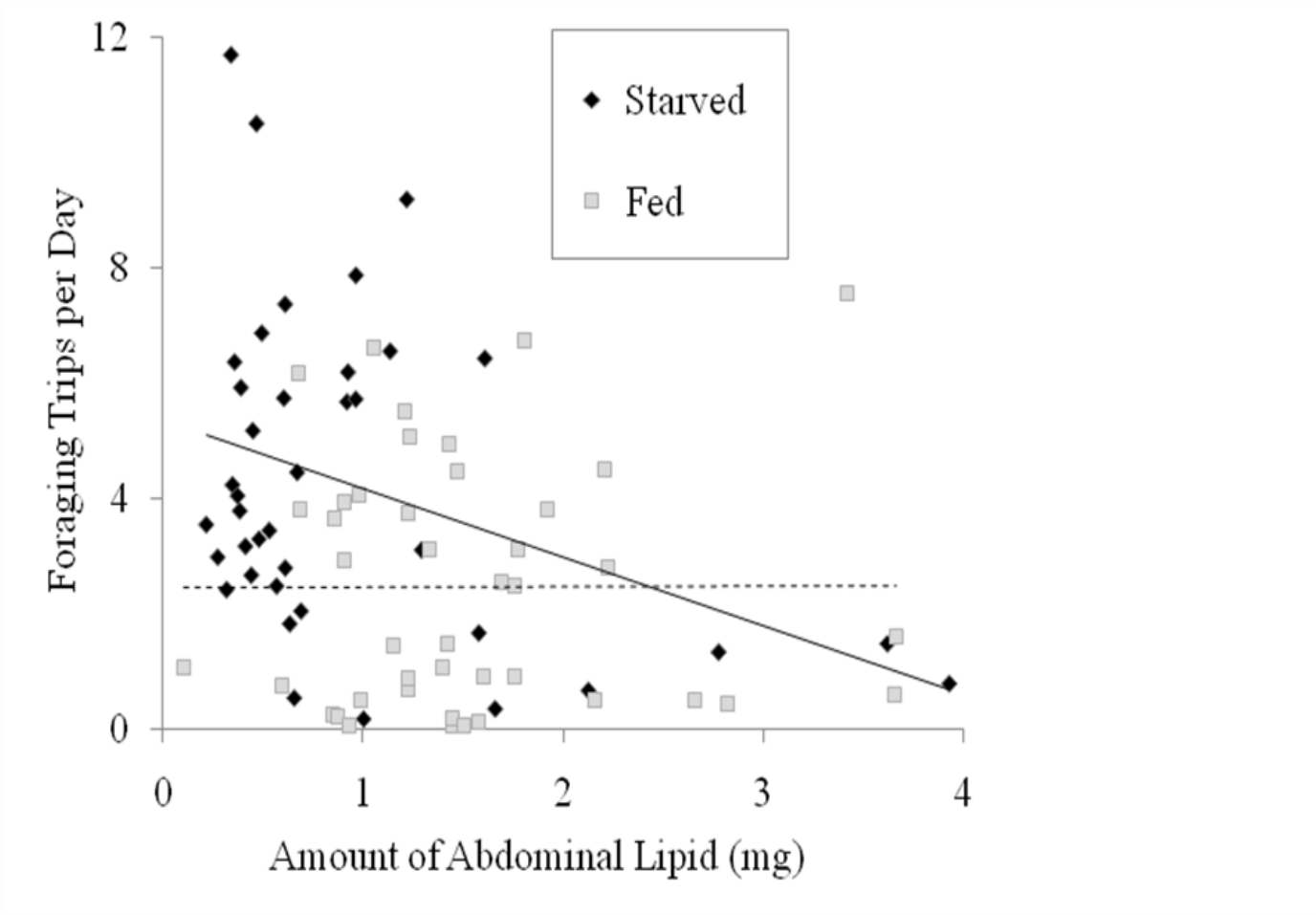


***Figure 5*** Relationship between abdominal lipid stores and foraging activity. This graph shows a strong negative relationship between abdominal lipid and foraging activity for starved colonies (Pearson correlation: R= -0.51, $t_{45}$=-4.02, $P$<0.001) while there is no observed correlation for fed colonies (Pearson correlation: R= 0.002, $t_{41}$=0.013, $P$>0.05).

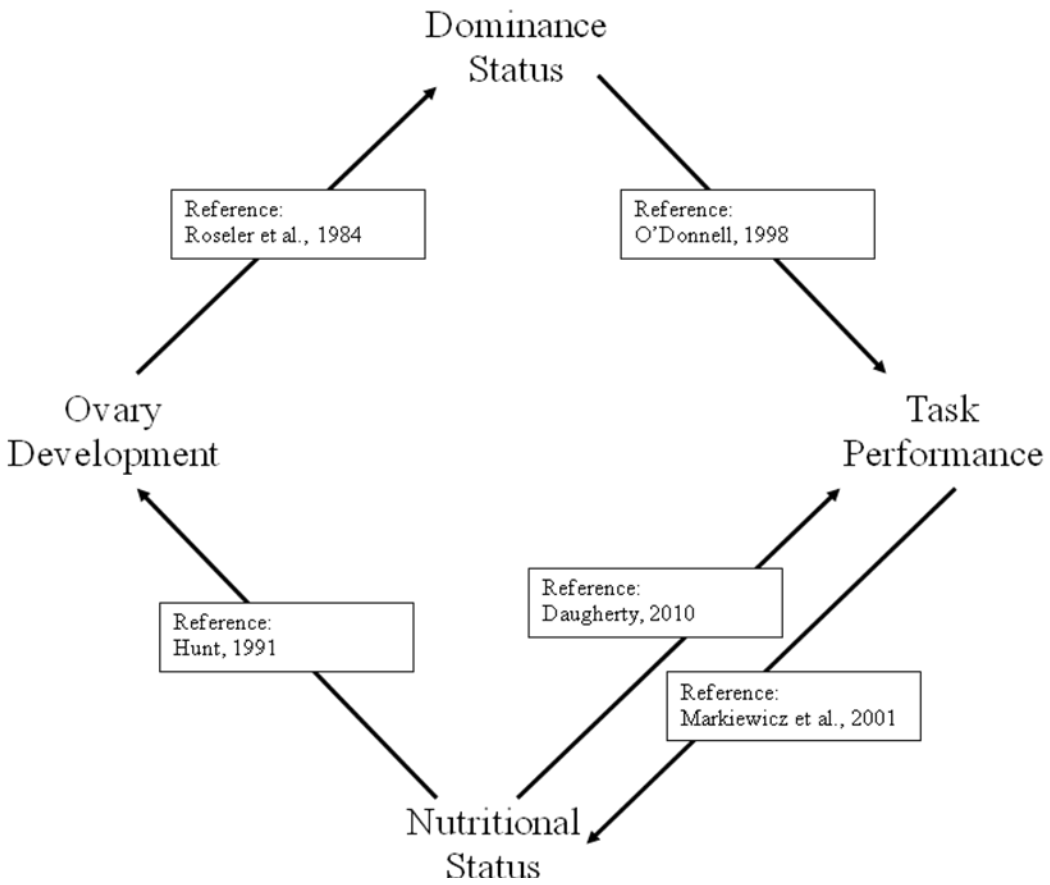


***Figure 6*** The relationship between nutrition, behavior, and reproductive physiology as proposed by Markiewicz and O'Donnell (2001). This figure includes my contribution to this model in which nutrition also has an effect on task performance.

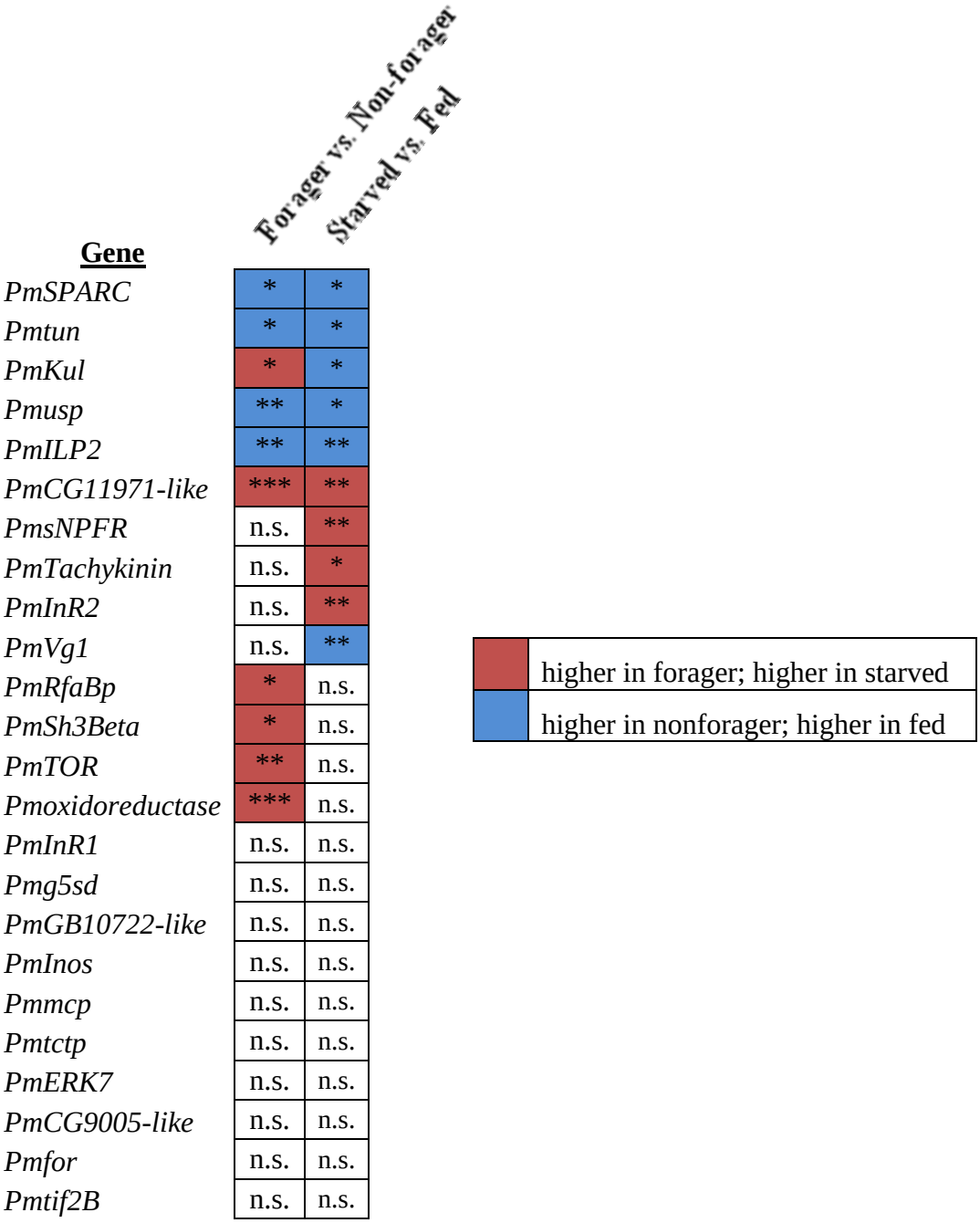


***Figure 7*** Genes associated with foraging or nutrition. Shown are the 24 genes analyzed in this study. The color indicates the direction of regulation: red, upregulated in Field-Reared Foraging Workers (FF) or Starved (LS); blue, upregulated in Field-Reared Non-foraging Workers (FNF) or Fed (LF); white, not significantly different. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; n.s., not significant.

**Chapter 7: References**

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